Selection of Dose Levels for Mammalian *in Vivo* Tier 1 Assays: Discussion Points

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Background

- Tier 1 *in vivo* assays will provide information on the potential of agents to interfere with the endocrine system.
- These screens should employ high dose levels since detailed dose response assessment is not a requirement and some false positives are acceptable.
- A NOAEL is not required.

Selection of highest dose level to be tested

- EDSTAC recommended the concept of a MTD, and if necessary other dose levels at a fraction of the MTD.
- Concept of a limit dose endorsed (1g/kg/d po).
- Dose selection based on all information available for test agent:
  - Previous toxicology data.
  - Predicted SAR.
  - Member of chemical class, etc.

Selection of highest dose level to be tested -2

- Default Approach
  - Up to 10% mortality.
- Mortality deemed by most investigators too severe for an endocrine screen.
  - Dose level should be high enough such that agents testing negative → hold box. Negative data considered adequate.
  - Dose level should produce some overt systemic toxicity to confirm a "stressed system".
  - If limit dose not used, second level should be biased towards upper end of dose response. Half the highest level is suggested.

Proposed high dose level assignment -1

- Number of toxicological indices available including body weight, food consumption and clinical signs.
- As guidance, recommend a 10% decrease in terminal bodyweight compared to control.
  - Less than default
  - High enough to account for potential estrogen effects on appetite

Proposed high dose level assignment -2

- Dose level selected is not a "bright line".
  - Would not reject a study for only achieving a 9% decrease.
  - Would not dismiss effects noted at 11% as "exceeding MTD".
  - Dose level selection based on individual chemical and in some instance body weight would not be appropriate.
  - No substitute for good judgment by investigator.

Pilot studies

- All *in vivo* mammalian Tier 1 assays conducted in the rat. Many other variables:
  - Gender, age, route of administration, duration of exposure, castration status.
- Dose levels may be selected based on previous relevant toxicological information.
- Some agents with little, or no data will require a pilot study

Pilot studies -2

- Recommendations
  - Take a stepwise approach.
  - Use the route of exposure needed for assay.
  - Maximally for duration of study.
  - Minimize use of animals (max 4-5).
  - Use up/down methodology.
  - For short duration assays sensible to take target organs (e.g. uterus).
  - Increases in weights of pharmacologically important organs much easier to interpret than decreases accompanied by bodyweight reductions.

Validation efforts

- Dose setting guidance and validation of protocols can occur in parallel.
- Protocol validation with given dose levels:
  - Can labs produce comparable results?
  - Is the protocol robust and transferable?
  - Is the lab competent to undertake the assay?
- Could run a test "unknown" to include dose setting prior to Tier 1 assay.
  - Test of guidance information and the investigators.
  - Dose levels should be qualitatively similar between labs.
  - Ability to discern response should be fulfilled.